CLAIMS

1. A catalytic nucleic acid nanostructure comprising a DNAzyme/RNAzyme and a polymerase-responsive element which comprises an internal hairpin structure and is elongated in the presence of the polymerase, wherein the elongation inhibits catalytic activity of the DNAzyme/RNAzyme.

2. The catalytic nucleic acid nanostructure of claim 1, wherein the DNAzyme/RNAzyme is selected from the group comprising:

a ribonuclease, such as ribonuclease 8-17, ribonuclease 10-23 or Dz10-66 deoxyribozyme;

a deoxyribonuclease, such as 10MD5 deoxyribozyme or 9NL27 deoxyribozyme;

a peroxidase, such as G-quadruplex Hemin;

an enzyme with ligation activity, such as E47 deoxyribozyme;

a phosphatase, such as 14WM9 deoxyribozyme;

an amide hydrolyser, such as AmideAm1 deoxyribozyme; and

an RNA branching enzyme, such as 9F7 deoxyribozyme or 7S11 deoxyribozyme.

3. The catalytic nucleic acid nanostructure of claim 1 or 2, wherein the DNAzyme/RNAzyme activity is a peroxidase activity.

4. The catalytic nucleic acid nanostructure of claim 3, wherein the DNAzyme/RNAzyme is G-quadruplex Hemin.

5. The catalytic nucleic acid nanostructure of claim 4, wherein the G-quadruplex Hemin DNAzyme comprises the nucleotide sequence set forth in SEQ ID NO: 1.

6. The catalytic nucleic acid nanostructure of claim 4, wherein the polymerase-responsive element comprises the nucleic acid sequence set forth in SEQ ID NO: 10.

7. The catalytic nucleic acid nanostructure of claim 4, wherein the G-quadruplex Hemin DNAzyme comprises the nucleotide sequence set forth in SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9.

8. The catalytic nucleic acid nanostructure of any one of claims 2 to 7, wherein activity of a peroxidase substrate is detected by different modalities, including but not limited to colorimetric, fluorescence, electrochemical or luminescence means.

9. A method of detecting polymerase activity in a test sample, comprising the steps of:

(a) providing a test sample;

(b) providing a composition comprising a catalytic nucleic acid nanostructure of any one of claims 1 to 8;

(c) contacting the sample in a) with the composition in b) in the presence of DNAzyme/RNAzyme substrate and, optionally, signal development reagents;

(d) detecting signal development, wherein the intensity of signal is inverse to the amount of polymerase activity in the sample.

10. A method of detecting target molecules in a sample, comprising the steps of:

(a) providing a test sample;

(b) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer adapted to recognize said target molecule in the sample;

(c) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer having a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to and forms a duplex with, a portion of an inverter oligonucleotide, wherein the inverter oligonucleotide is adapted to recognize said target molecule in the sample with a higher affinity than the variable duplex region;

(d) contacting the test sample with the composition of (b) or (c), wherein target molecule binding to:

(i) the recognition sequence region of the aptamer in (b) promotes the formation of a stable aptamer-DNA polymerase enzyme complex, thereby inhibiting DNA polymerase enzyme activity; or

(ii) the inverter oligonucleotide in (c) destabilizes the recognition nanostructure, thereby releasing the DNA polymerase enzyme from inhibition by the DNA aptamer;

(e) providing a catalytic nucleic acid nanostructure of any one of claims 1 to 8;

(f) contacting the nanostructure from step (b) or (c) in the presence of DNAzyme/RNAzyme substrate and, optionally, signal development reagents;

(g) detecting signal development, wherein the intensity of signal indicates;

(i) presence of target molecule in the sample when using composition (b); or

(ii) the absence of target molecule in the sample when using composition (c).

11. A method of detecting target nucleic acids in a sample, comprising the steps of:

(a) providing a sample comprising nucleic acid;

(b) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer having a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to a target nucleic acid in the sample; or

(c) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer and an inverter oligonucleotide, wherein the aptamer has a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to, and forms a duplex with, a portion of the inverter oligonucleotide, wherein the inverter oligonucleotide is at least one nucleotide longer than the aptamer-inverter duplex and has more than 10 nucleotides complementary to a target nucleic acid in the sample;

(d) contacting the sample comprising nucleic acid with the composition of (b) or (c), wherein target nucleic acid binding to:

(i) the variable sequence region of the aptamer in (b) promotes the formation of a stable aptamer-DNA polymerase enzyme complex, thereby inhibiting DNA polymerase enzyme activity; or

(ii) the inverter oligonucleotide in (c) destabilizes the recognition nanostructure, thereby releasing the DNA polymerase enzyme from inhibition by the DNA aptamer;

(e) providing a catalytic nucleic acid nanostructure of any one of claims 1 to 8;

(f) contacting the nanostructure from step (d) in the presence of DNAzyme/RNAzyme substrate and, optionally, signal development reagents;

(g) detecting signal development, wherein the intensity of signal indicates;

(i) presence of target nucleic acid in the sample when using composition (b); or

(ii) the absence of target nucleic acid in the sample when using composition (c).

12. A device comprising a catalytic nucleic acid nanostructure of any one of claims 1 to 8 immobilized on a surface.

13. The device of claim 12 comprising:

(i) composition of b) or composition of c) comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, as defined in claim 10, at a first location;
(ii) catalytic nucleic acid nanostructure of any one of claims 1 to 8, attached at a second location; and
(iii) an intermediate stage for mixing of said detection nanostructures with sample nucleic acid to release active enzyme to said second location.

14. The device of claim 12 or 13, selected from a group comprising a microfluidic device and a lateral flow device.

15. The device of any one of claims 12 to 14, comprising an electrode.

16. A nucleic acid detection kit comprising;
(a) a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer having a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to a target nucleic acid; and/or
(b) a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer and an inverter oligonucleotide, wherein the aptamer has a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to, and forms a duplex with, a portion of the inverter oligonucleotide, wherein the inverter oligonucleotide is at least one nucleotide longer than the aptamer-inverter duplex and has more than 10 nucleotides complementary to a target nucleic acid;
(c) a catalytic nucleic acid nanostructure of any one of claims 1 to 8; optionally
(d) DNAzyme/RNAzyme substrate and, optionally,
(e) signal development reagents.

17. A molecule detection kit comprising;

(a) a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer having a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to and forms a duplex with, a portion of the inverter oligonucleotide, wherein the inverter oligonucleotide is adapted to recognize said target molecule in the sample with a higher affinity than the variable duplex region;

(b) a catalytic nucleic acid nanostructure of any one of claims 1 to 8; optionally

(c) DNAzyme/RNAzyme substrate and, optionally,

(d) signal development reagents.